GERMINATION AND HYPHAL GROWTH OF VAM FUNGI DURING AND AFTER STORAGE IN SOIL AT FIVE MATRIC POTENTIALS

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Summary—Four vesicular-arbuscular mycorrhizal (VAM) fungi, *Gigaspora margarita* Becker & Hall, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *Glomus intraradices* Schenck & Smith, and *Acaulospora longula* Spain & Schenck, were studied to characterize the variety of responses of VAM fungi to storage. Spores were stored at 23°C for up to 4 months in soil at several matric potentials [ψm], then removed and exposed for 1 month in soil at field capacity to induce germination. Each species studied has a different response to storage duration and moisture availability, reflecting the complexity of the problem of storage of VAM fungi in soil. Net germinability, the difference in percentage germination between that which occurred in storage and germination after the subsequent month at field capacity, was effectively zero for *Gi. margarita*. Hyphae continued to grow and additional germ tubes were produced upon removal from storage. *Gi. margarita* spores which produced one germ tube per spore during storage in moist soil subsequently produced less root colonization of *Paspalum notatum* than spores stored in drier soil. Net germinability of spores of *G. intraradices* increased with decreasing storage ψm and was independent of storage duration. Upon removal from storage, preexisting hyphae resumed growth. Net germinability of spores of *G. mosseae* was inversely proportional to duration of storage and independent of ψm. Spores which germinated in storage did not resume growth upon removal from storage. Net germinability of *A. longula* increased with duration of storage and was independent of moisture availability during storage, indicating a dormant period.

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi are most commonly maintained in pot culture with a plant. This method requires much space and labor and may not preserve the genetic variability of the original population collected from the field. Even so, storage of VAM fungus spores and infected root pieces in pot culture soil, though bulky, may be the most reliable and broadly applicable method of preservation available (Siqueira *et al.*, 1985). VAM fungi in excised roots can remain infective for *ca* 2 years (Tommerup, 1981). Spores of *Glomus fasciculatum* have remained viable after storage for 4 years in dry soil (Ferguson and Woodhead, 1982). Fine sand soil containing spores of *Glomus intraradices* remained infective after 175 days of storage at 1% moisture and temperatures from 1 to 21°C (Nemec, 1987). Storage of dried pot culture soil with spores at room temperature has also been used for *Glomus clarum* (Louis and Lim, 1988).

Knowledge of the response of VAM fungus spores to factors limiting viability in storage is essential to the development of protocols for the storage of pot-culture soil. Factors limiting survival of spores in storage include temperature, moisture availability in the soil, and duration of storage (Tommerup, 1983, 1987; Nemec, 1987). Our objective was to characterize the variety of responses of spores of VAM fungi to exposure to soil at room temperature at several matric potentials. We report spore germination, germ tube production, and hyphal growth of four species of VAM fungi in storage and after a subsequent exposure of 1 month in moist soil. The effect of storage upon the ability of one fungus to colonize plant roots also was examined.

MATERIALS AND METHODS

Experiment I

Spores of four species of VAM fungi were isolated from pot cultures with *Paspalum notatum* Flugge and *Medicago sativum* L. as plant hosts. Fungi used were: *Glomus mosseae* [International Culture Collection of VA Mycorrhizal Fungi (INVAM) isolate number 156] (Nicol. and Gerd.) Gerdemann and Trappe, *G. intraradices* (INVAM 208) Schenck and Smith, *Gigaspora margarita* [INVAM 185] Becker and Hall, and *Acaulospora longula* (INVAM 316) Spain and Schenck. Pot cultures ranged in age from 7 months (*Gi. margarita*) to 23 months (*A. longula*). Cultures with spore populations sufficient to supply spores for the entire experiment were selected to ensure a homogeneous source of spores.

Spores were isolated from soil by wet sieving (Gerdemann and Nicolson, 1963) and centrifugation (Jenkins, 1964) and placed on membrane filters (0.45 μm pore size, 2.5 cm dia). Three filters per soil moisture per sample period per VAM fungus species were prepared ($5 \times 5 \times 4$, respectively). The mean number of spores per filter ($\pm$SEM) was:

Gi. margarita, 23.0 ± 1.0; *G. mosseae*, 32.2 ± 1.4; *G. intraradices*, 33.2 ± 1.4; and *A. longula*, 40.4 ± 2.1. Filters were folded and placed in tissue specimen bags (Shandon Southern Instruments, Inc., Sewickley, Pa.). Spores have been shown to germinate equally well when exposed between membrane filters in soil or directly in soil (Tommerup, 1983).

The membrane filters in specimen bags were buried in plastic bags filled with pasteurized (70°C for 2 h) soil at five matric potentials [ψm]: −0.01, −0.05, −0.50, −1.30 and −2.20 MPa. Soil water contents at various ψm were determined using a ceramic plate extractor (No. 1500, Soil Moisture Equipment Co., Santa Barbara, Calif.). *G. mosseae*, *A. longula* and *Gi. margarita* spores were originally cultured, stored and germinated in Arredondo fine sand soil (loamy, siliceous, hyperthermic Grossarenic Paleudult). *G. intraradices* spores were originally cultured, stored and germinated in a mixture of Arredondo fine sand soil and calcined clay ("Emathlite", Mid-Florida Mining Co., Lowell, Fla, U.S.A.; 1.4:1.0 v/v). Bags were sealed and stored in the dark at 23°C. Samples were removed at 2, 4, 8 and 16 weeks to assay spore germination.

Spores were germinated by placing the specimen bags in a tray containing a layer of pasteurized soil and then covering them with an additional 1 cm of soil. The soil was watered with deionized water and maintained at field capacity and room temperature. After 1 month, the filters were removed and spores and hyphae were stained with 0.05% Trypan Blue. Spores were considered germinated if germ tubes longer than thick, darkly stained, residual attached hyphae were present. Total length of VAM fungus hyphae on membrane filters removed at 16 weeks was determined by the line-intersect method (Newman, 1966). Hyphae intersecting parallel lines 1 mm apart were counted and data expressed as length of hyphae per germinated spore.

A second sample of spores was removed from storage at 4 months. These spores were stained immediately with Trypan Blue to assay germination which occurred during storage. Length of hyphae per germinated spore was calculated as above.

Experiment II

A second experiment was conducted to characterize the germination of spores of *Gi. margarita* and to determine if germination during storage subsequently affected the ability of the fungus to colonize roots of *P. notatum*. Spores first were isolated from moist, fresh pot-culture soil (Arredondo fine sand) and placed in the germination assay outlined above. The remainder of the soil was allowed to air dry. Water contents were determined gravimetrically on subsamples removed at each of four stages of drying (ψm = −0.0044, −0.0052, −0.0075 and −0.675 MPa; 14.5, 12.0, 7.9 and 3.8% water [w/w] respectively). The remainder of soil removed at each stage was placed in a plastic bag for exposure at 23°C. This experiment differed from experiment I because spores were exposed *in situ* rather than on membrane filters.

Soil was removed from the bags after 3, 7.5, 12 and 19 weeks. Spores were isolated by wet sieving and centrifugation, a process which broke hyphae and left stubs of germ tubes and hyphal attachments. Spores were placed on three membrane filters and germinated as above. Healthy or unhealthy appearing spores were not discriminated for or against at the initiation of the assay so samples reflected changes in the population of spores present in the soil at the beginning of the experiment. At the conclusion of the germination assay, spores were scored for germination, hyphal length per germinated spore and intact and broken germ tubes per spore.

The last sample of soil + spores of *Gi. margarita* was removed at 25 weeks and used in a colonization test. A 5 g (adjusted dry wt) sample of soil of each ψm was mixed into 175 g (dry wt) of pasteurized Arredondo fine sand soil and placed in a conical plastic pot ("conetainer", Ray Leach Cone-Tainer Nursery, Canby, Ore.). Three replications per original incubation ψm were prepared. Two *P. notatum* seedlings were transplanted into each pot and grown under controlled conditions: 14–10 h day–night; 28–21°C; and 600–800 μmol m^{-2} s^{-1} photosynthetic photon flux density for 2 weeks. Entire root systems were cleared, stained (Phillips and Hayman, 1970) and assayed for percentage root length colonized and number of penetration points.

Data analysis

Net germination was defined as the difference in percentage germination between that which occurred in storage and after the subsequent month at field capacity. Data were analyzed using analysis of variance or linear regression where applicable. Percentage germination and colonization were analyzed using arcsin transformed data.

RESULTS

Experiment I

Thirty-seven percent of the *Gi. margarita* spores isolated from pot culture germinated when exposed to soil at field capacity (time = 0, Fig. 1). Spores showed a strong tendency to germinate in storage at all ψm studied (time = S, Fig. 1). Approximately 85% of the spores germinated when stored on membrane filters in soil of ψm = −0.01 MPa and 45% in ψm = −2.2 MPa. Germinated spores produced more hyphae in storage at −0.01 MPa than at −2.2 MPa (Table 1). Spores which germinated in storage approximately doubled their length of hyphae upon exposure for 1 month in the germination assay. This was possible due to both continued growth from previously-formed hyphae and the production of new germ tubes. These responses were greater at −2.2 MPa than at other moisture contents tested. Due to the germination of *Gi. margarita* spores in storage and their ability to resume growth upon removal from storage, survival was independent of both storage duration and soil ψm and net germination was effectively zero.

Forty-eight percent of the *G. intraradices* spores isolated from pot culture germinated when exposed to soil at field capacity (time = 0, Fig. 2). More than 70% germinated while in storage on membrane filters at −0.01 and −0.05 MPa, but only 1%

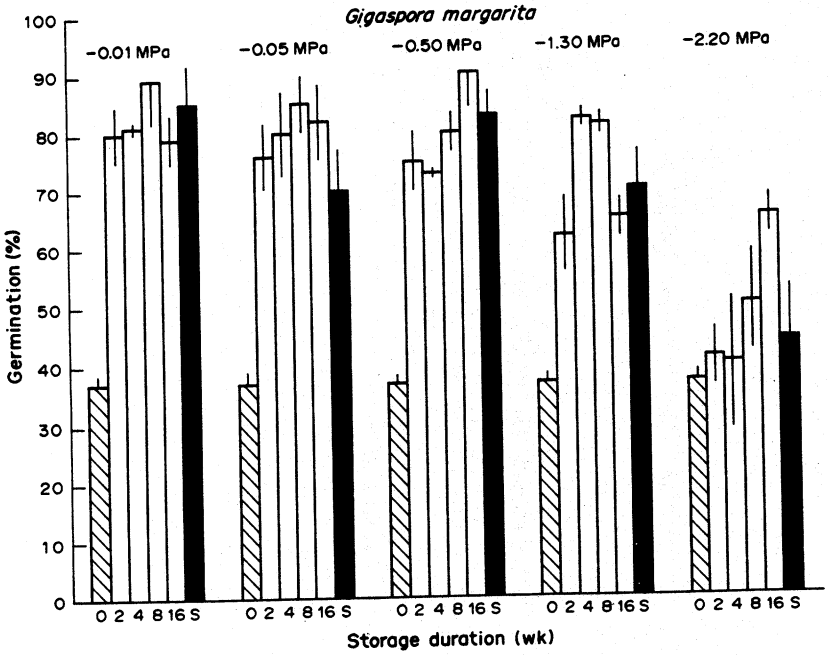

Fig. 1. Germination of spores of *Gi. margarita* in storage at 23°C in Arredondo fine sand with various matric potentials [ψm]. Time 0 = germination upon isolation from original pot culture; germination at 2, 4, 8 and 16 weeks = percentage of spores removed from storage at these times and assayed as having germinated after 1 month's exposure in soil at field capacity; S = germination of spores while in storage, assayed immediately after 4-months storage and believed to have occurred during the first weeks of storage. Means of three samples of spores ± SEM.

germinated in storage on membrane filters at −2.2 MPa (time = S, Fig. 2). Spores which germinated in storage produced an additional 82–106% hyphal length (−0.05 and −0.01 MPa respectively) upon exposure for 1 month in the germination assay (Table 2). Production of a second germ tube was not evident, so the additional hyphal growth resulted from regrowth from original germ tube hyphae. Net germinability of spores of *G. intraradices* stored at room temperature was inversely related to soil ψm and was independent of time under the conditions of this experiment (net percentage germination = −27.70(MPa) + 13.48; $r = -0.88$). For example, *G. intraradices* spores exhibited a net germination of 46% after 4 weeks storage at −0.050 MPa (76% total germination minus 30% germination in storage) and 73% net germination after 4 weeks storage at −1.30 MPa (75% total germination minus 2% germination in storage).

Fifty-two percent of the *G. mosseae* spores isolated from pot culture germinated when incubated in soil at field capacity (time = 0, Fig. 3). Germination of spores in storage declined from 30% at −0.01 MPa to 3–4% at −0.5 to −2.2 MPa (time = S, Fig. 3). Germinated spores produced significantly more hyphae while in storage at −0.01 MPa than at ψm of −0.5 to −2.2 MPa (Table 2). These spores did not produce additional hyphae when removed and exposed at field capacity in the germination assay. This, and their vacuolated, discolored appearance

Table 1. Hyphal length and number of germ tubes produced by spores of *Gi. margarita* stored for 4 months in soil at various matric potentials [ψm] and upon 1 month of further exposure in soil at field capacity (germination assay), experiment I[a]

	Hyphae (mm germinated spore^{-1})		Germ tubes spore^{-1}	
ψm (MPa)	Storage	Germ. assay	Storage	Germ. assay
−0.01	39B	54A	1.1A	1.2A
−0.05	22A	58A	1.0A	1.1A
−0.50	25A	43A	1.0A	1.1A
−1.30	24B	52A	1.0A	1.1A
−2.20	20B	97A	1.0B	1.3A
Regression[b]	NS	**	NS	**

[a]Each number represents the mean of three observations. Numbers for a comparison between storage and germination assay within a ψm followed by the same letter are not significantly different ($\alpha = 0.05$, Duncan's multiple range test).

[b]Significance of linear regression of each dependent variable (hyphal length or germ tubes) with ψm (MPa) as the independent variable. NS, not significant; **, significant ($P < 0.05$).

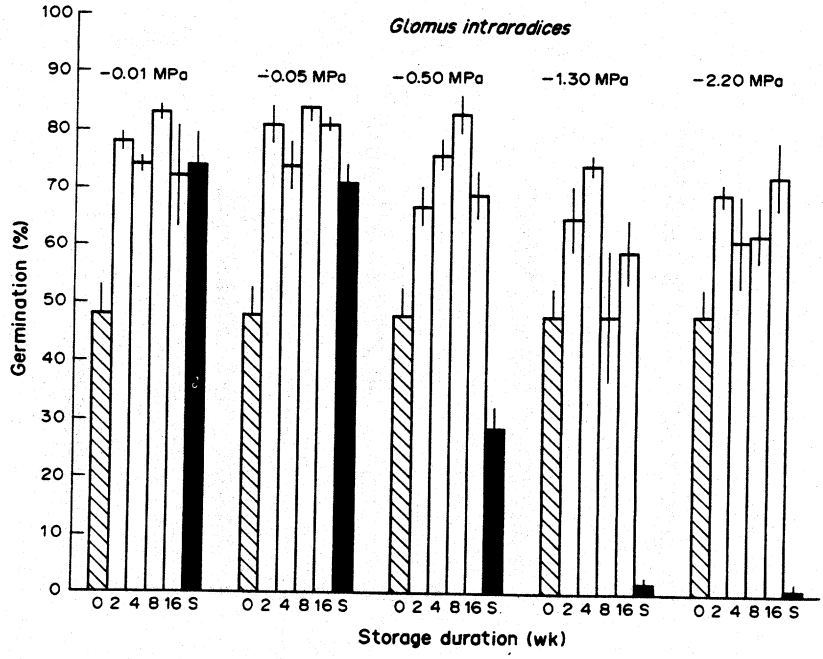

Fig. 2. Germination of spores of *G. intraradices* after storage at 23°C in Arredondo fine sand-calcined clay soil mix with various ψm. See legend of Fig. 1.

suggested they were dead. Germination of spores of *G. mosseae* upon removal from storage and exposure at field capacity was independent of storage ψm and inversely proportional to duration at those ψm in which spores did not germinate significantly in storage [net percentage germination $= -1.87$ (weeks) $+ 28.88$; $r = -0.79$]. For example, *G. mosseae* spores exhibited a net germination of 23% after 2 weeks storage at -0.50 MPa (27% total germination minus 4% germination in storage) and only 4% net germination after 16 weeks at that ψm (8% total germination minus 4% germination in storage).

Twenty-nine percent of the *A. longula* spores isolated from pot culture germinated when exposed to soil at field capacity (time = 0, Fig. 4). Germination of spores in storage declined from 19% at ψm $= -0.01$ MPa to 5–7% at ψm $= -0.5$ to -2.2 MPa (time = S, Fig. 4) and spores produced few hyphae (Table 2). Otherwise, the effect of

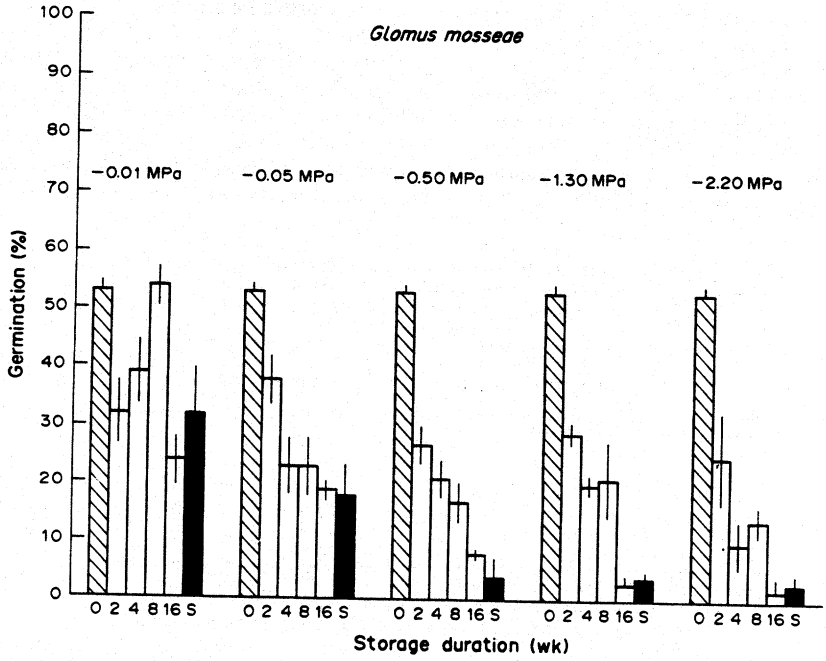

Fig. 3. Germination of spores of *G. mosseae* after storage at 23°C in Arredondo fine sand soil with various ψm. See legend of Fig. 1.

Table 2. Length of hyphae produced by spores of VAM fungi while in storage for 4 months in soils at various matric potentials (ψm) and upon 1 month of further exposure in soil at field capacity (germination assay), experiment I[a]

Species	ψm (MPa)	Hyphae (mm germinated spore^{-1}) Storage	Germination assay
G. intraradices	−0.01	5.2B	10.8A
	−0.05	5.3A	9.6A
	−0.50	10.0A	15.5A
	−1.30	3.4B	12.7A
	−2.20	1.6B	10.7A
Regression		*	NS
G. mosseae	−0.01	19.5A	10.1A
	−0.05	12.7A	2.9A
	−0.50	3.5A	5.0A
	−1.30	1.8A	0.5A
	−2.20	0.5A	0.5A
Regression		**	**
A. longula	−0.01	0.9B	3.7A
	−0.05	0.3A	2.6A
	−0.50	0.7A	3.2A
	−1.30	0.1B	3.4A
	−2.20	0.5B	3.8A
Regression		NS	NS

[a]Numbers are the means of three observations. Compare numbers between columns for a particular species × ψm combination only. Statistics as in Table 1, * $P < 0.10$.

ψm upon survival and germinability was minor relative to the effect of storage duration [net percentage germination = 2.84 (weeks) + 13.77; $r = 0.87$]. For example, *A. longula* spores exhibited net germination of 19% after 4 weeks storage at −2.2 MPa (26% total germination minus 7% germination in storage) and 73% net germination after 16 weeks at that ψm (80% total germination minus 7% germination in storage). Length of storage enhanced germination of *A. longula* spores upon removal from storage at all soil moisture tensions tested.

Experiment II

Nearly 90% of the spores of *Gi. margarita* survived to produce new germ tubes after 19 weeks of storage *in situ* at room temperature at all ψm studied (Table 3). With each moisture tension, germination appeared to be lower after 3–7.5 weeks exposure than after 19 weeks. Germination after 7.5 weeks exposure was significantly lower than after 19 weeks. Hence, a quadratic equation was used to analyze the data. Germination after 7.5 weeks exposure was significantly lower than after 19 weeks for all ψm studied.

The amount of hyphae produced by each germinated *Gi. margarita* spore during the germination assay was affected little by time ($r = -0.210$, $P > 0.10$) or moisture tension during incubation ($r = 0.354$, $P > 0.05$) (Table 3). The amount of hyphae produced after an exposure of 19 weeks was not statistically different from that produced by spores not subjected to air drying and storage.

The number of new germ tubes produced by *Gi. margarita* during the germination assay tended to increase with duration of storage and moisture tension during storage (Table 3). New germ tube production was affected by ψm at the 12 and 19 week sample periods and by storage duration at −0.0044 and −0.0075 MPa. Total germ tubes, new plus those produced in storage and broken upon isolation from the soil, increased with duration of exposure (Table 3).

Inoculum of *Gi. margarita* stored at room temperature for 25 weeks was able to colonize *P. notatum* seedlings within 2 weeks of inoculation (Table 4). Inoculum stored at −0.0044 MPa produced fewer penetration points per length of root than other inocula, indicating lower infectivity even though spore numbers and percentage germination were the same for all inocula after 19 weeks (Table 3).

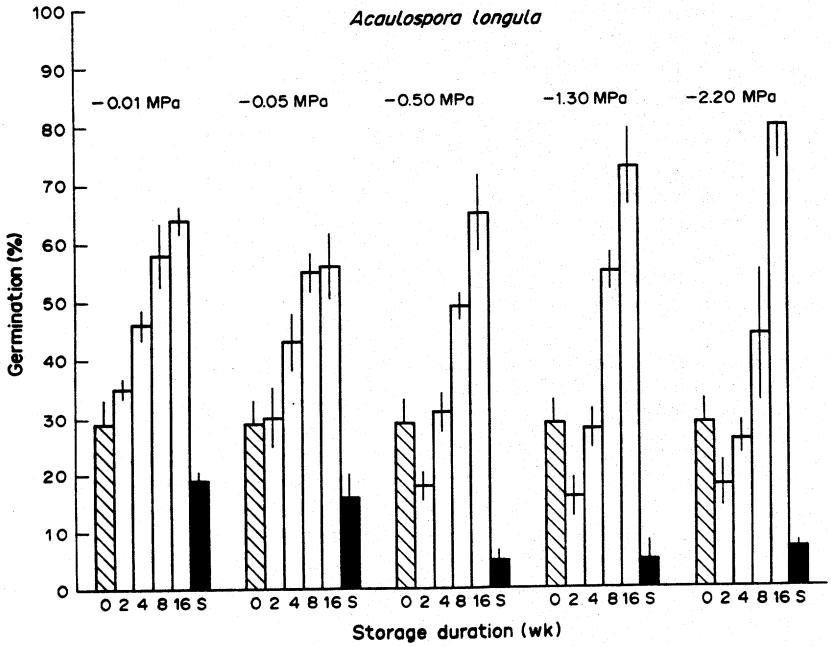

Fig. 4. Germination of spores of *A. longula* after storage at 23°C in Arredondo fine sand soil with various ψm. See legend of Fig. 1.

Table 3. Percentage germination and hyphal growth and germ tube production per germinated spore for *Gi. margarita* stored in soil at various matric potentials and subsequently exposed 1 month in moist soil, experiment II[a]

ψm (MPa)	Storage (weeks)	Germination percentage	Hyphae (mm germinated spore^{-1})	Germ tubes	
				New	Old
−0.0044	0.0	76 ± 4	95 ± 11	1.01 ± 0.01	1.01 ± 0.01
	3.0	67 ± 4	109 ± 7	1.14 ± 0.01	1.14 ± 0.01
	7.5	42 ± 4	83 ± 8	1.02 ± 0.02	1.08 ± 0.02
	12.0	68 ± 5	111 ± 8	1.07 ± 0.02	1.17 ± 0.05
	19.0	88 ± 4	74 ± 12	1.32 ± 0.08	2.03 ± 0.36
Regression[b]		**	NS	**	**
−0.0052	0.0	76 ± 4	95 ± 11	1.01 ± 0.01	1.01 ± 0.01
	3.0	67 ± 6	123 ± 7	1.11 ± 0.05	1.11 ± 0.05
	7.5	55 ± 8	97 ± 4	1.14 ± 0.07	1.14 ± 0.07
	12.0	65 ± 4	114 ± 13	1.06 ± 0.03	1.17 ± 0.03
	19.0	87 ± 2	81 ± 13	1.07 ± 0.02	1.31 ± 0.11
Regression[b]		**	NS	NS	**
−0.0075	0.0	76 ± 4	95 ± 11	1.01 ± 0.01	1.01 ± 0.01
	3.0	62 ± 6	126 ± 12	1.04 ± 0.02	1.04 ± 0.02
	7.5	46 ± 3	147 ± 14	1.05 ± 0.03	1.05 ± 0.03
	12.0	64 ± 2	134 ± 11	1.03 ± 0.02	1.10 ± 0.02
	19.0	84 ± 4	86 ± 3	1.11 ± 0.04	1.24 ± 0.11
Regression[b]		**	**	**	**
−0.6750	0.0	76 ± 4	95 ± 11	1.01 ± 0.01	1.01 ± 0.01
	3.0	41 ± 5	125 ± 12	1.05 ± 0.05	1.05 ± 0.05
	7.5	47 ± 1	113 ± 25	1.09 ± 0.04	1.09 ± 0.04
	12.5	89 ± 2	119 ± 3	1.01 ± 0.01	1.02 ± 0.02
	19.0	90 ± 3	96 ± 12	1.11 ± 0.02	1.41 ± 0.07
Regression[b]		**	NS	NS	**
Regression[c]	3.0	**	NS	NS	NS
	7.5	NS	NS	NS	NS
	12.0	**	NS	*	**
	19.0	NS	NS	*	NS

[a]Each number represents the mean of three observations ± SEM.
[b]Significance of linear regression of each dependent variable with weeks + (weeks)^2 of storage as independent variables. NS, not significant; ** significant ($P < 0.05$).
[c]Significance of linear regression of each dependent variable using ψm (MPa) as the independent variable, i.e. within a storage duration across ψm. NS, not significant; *$P < 0.10$, **$P < 0.05$.

DISCUSSION

Spores of *Gi. margarita* germinated in storage at all moisture contents in experiment I, possibly due to hydration of spores during wet sieving and placement on moist membrane filters prior to storage. Rapid hydration of *Glomus caledonium* spores has been reported (Tommerup, 1984b). After rehydration, spores of *G. caledonium* and *A. laevis* would germinate equally well in soil with ψm as low as −1.58 MPa as in wetter soil (Tommerup, 1984b). Germination of rehydrated spores in dry soil may have an adverse effect upon reserves of VAM fungi in soil (Tommerup, 1984b; Thompson, 1987).

Spore isolation and placement on membrane filters prior to incubation in dry soil in experiment I, though previously shown to have no effect upon the change of dormant spores to a quiescent state (Tommerup, 1983), also may have stimulated germination by leaching potential inhibitors of germination from the spore or spore wall of *Gi. margarita* and *G. mosseae*. Washing *G. caledonium* spores has been shown to enhance germination (Tommerup, 1985), but washing *Gi. margarita* spores with water did not stimulate germination nearly as well as surface sterilization with sodium hypochlorite (Sward, 1981). In experiment II where *Gi. margarita* spores were dried *in situ*, and hence, no leaching occurred, there was no firm evidence of germination in storage until the 19-week harvest in which there were significantly more old, broken germ tubes presumed to have been produced during the storage period. Even so, at ψm = −0.675 MPa in experiment II, a maximum of 27% of spores germinated in storage after 19 weeks (0.3 old germ tubes per spore × 89.7% germination) (Table 3) compared to 83% germination in storage at −0.5 MPa in experiment I (Fig. 1). The data show that hydration or leaching of potential inhibitors of germination were significant factors in the storage of *Gi. margarita* in experiment I.

The result of germination in storage, whether due to the soil ψm being conducive to germination or previous hydration or leaching of spores, differed among the VAM fungi tested. Germination of *G. mosseae* spores in storage yielded spores which were unable to grow when removed from storage.

Table 4. Total root length and root colonization of *Paspalum notatum* seedlings by *Gi. margarita* after 2 weeks of growth. Inocula were stored 25 weeks in soil at four matric potentials (ψm) at 23°C[a]

Storage ψm (MPa)	Total root length (cm)	VAM fungus colonization[b]	Penetration points cm^{-1}
−0.0044	282 ± 30	8.3 ± 2.2	0.19 ± 0.03
−0.0052	172 ± 20	15.6 ± 2.2	0.52 ± 0.06
−0.0075	233 ± 18	21.5 ± 2.0	0.55 ± 0.08
−0.6750	278 ± 62	13.8 ± 1.0	0.48 ± 0.02
Regression[c]	NS	*	**

[a]Each number represents the mean of three observations ± SEM.
[b]Percentage of root length.
[c]Significance of linear regression of each dependent variable with ψm (MPa) as the independent variable. NS, not significant, *$P < 0.10$, and **$P < 0.05$.

Pregerminated spores of *Gi. margarita*, *G. intraradices* and *A. longula*, however, produced additional germ tubes or hyphal growth upon removal from storage and exposure to soil at field capacity. Their capacities to colonize roots, however, may have been affected. Spores of *Gi. margarita*, incubated 25 weeks in soil at the highest ψm studied in experiment II, showed decline in infectivity over spores incubated at the other ψm (Table 4). The data of the 19-week sample showed the same percentage germination and total hyphal lengths for spores, thus growth to potential host roots may not have been the limiting factor. Tommerup (1984a) found that *G. caledonium* and *A. laevis* were limited in their capacity to produce prepenetration and penetration structures when pregerminated up to 16 weeks before exposed to plant roots. The infectivity of pregerminated VAM fungal spores has been shown to decrease to 50% of maximal capacity 8 weeks after germination and to 0% at 16 weeks (Tommerup, 1981). Therefore, the combination of pregermination in moist soil and storage duration is detrimental to the potency of pot-culture inocula.

Species of VAM fungi undergo dormancy. A dormancy period was found here for *A. longula* and has been shown also for *A. laevis* (Tommerup, 1983). *Gi. margarita* and *G. intraradices* exhibited a dormancy period of *ca* 2 weeks in this study. Storage of dried inoculum of *G. clarum* at 25–30°C has been shown to enhance germination, suggesting a dormant period for this species (Louis and Lim, 1988). A 2–9 week dormancy period was estimated for field populations of *Gigaspora gigantea* (Gemma and Koske, 1988). Incubation in dry soil has been shown to release spores of VAM fungi from dormancy sooner than incubation in moist soil (Tommerup, 1983). This was not seen for species used here.

Each VAM fungus in experiment I exhibited a different response to storage duration and moisture availability. *Gi. margarita* displayed low net germinability throughout the experiment, independent of both storage duration and moisture. Germinability of *G. mosseae* was inversely proportional to duration. Survival of *G. intraradices* was dependent upon ψm and independent of time. Storage at room temperature released *A. longula* from dormancy.

Storage of spores of *G. intraradices* [208] and *A. longula* [216] in soil at 23°C and ψm less than -0.05 MPa appears to be an effective method to maintain and even enhance the germinability of these fungi. *G. mosseae* [156] may be stored at -0.5 to -2.2 MPa for 2 months only with significant loss of germinability. Storage of *Gi. margarita* [185] spores in soil at 23°C at ψm less than -0.01 MPa appears to be an ineffective method to ensure infectivity.

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